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# Adenylate Energy Charge in *Escherichia coli* During Growth and Starvation

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The value of the adenylate energy charge, [(adenosine triphosphate) + ½ (adenosine diphosphate)]/[(adenosine triphosphate) + (adenosine diphosphate) + (adenosine monophosphate)], in *Escherichia coli* cells during growth is about 0.8. During the stationary phase after cessation of growth, or during starvation in carbon-limited cultures, the energy charge declines slowly to a value of about 0.5, and then falls more rapidly. During the slow decline in energy charge, all the cells are capable of forming colonies, but a rapid fall in viability coincides with the steep drop in energy charge. These results suggest that growth can occur only at energy charge values above about 0.8, that viability is maintained at values between 0.8 and 0.5, and that cells die at values below 0.5. Tabulation of adenylate concentrations previously reported for various organisms and tissues supports the prediction, based on enzyme kinetic observations in vitro, that the energy charge is stabilized near 0.85 in intact metabolizing cells of a wide variety of types.

The adenine nucleotides, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP), stoichiometrically couple all of the metabolic sequences of a living cell. The amount of metabolically available energy that is momentarily stored in the adenylate system is linearly related to the mole fraction of ATP plus half the mole fraction of ADP; this parameter has been termed the *energy charge* of the adenylate pool (10). In terms of concentrations, the energy charge is thus [(ATP) + ½ (ADP)]/[(ATP) + (ADP) + (AMP)].

Response curves of activity as a function of energy charge have been obtained for several enzymes (for review, see reference 6). Regulatory enzymes from sequences in which ATP is regenerated are highly active at low levels of energy charge and decrease sharply in activity as the charge increases above a value of about 0.75. Regulatory enzymes from biosynthetic sequences or others that consume ATP exhibit very little activity at low levels of energy charge, and their activities increase sharply at charge values above about 0.75. Curves of these two types tend to intersect at an energy charge of about 0.85. If these response patterns observed in vitro reflect the behavior of the enzymes in vivo, the energy charge in a living cell must be rather strongly stabilized in a range near 0.85, since any tendency for the charge to fall would be resisted by the consequent increase in the rate of regeneration of ATP and decrease in the rates of sequences in which it is used, and a tendency to rise would be opposed by oppositely directed changes.

This paper reports values of energy charge in *Escherichia coli* cells during growth and under conditions of nutritional deficiency. Published estimates of adenine nucleotide concentrations in vivo are also tabulated and shown to be generally consistent with the prediction that the energy charge should be stabilized at values around 0.8 to 0.9.

## MATERIALS AND METHODS

**Growth conditions.** *E. coli* B was grown in a medium containing (per liter): 5 g of $KH_2PO_4$, 13 g of $K_2HPO_4$, and 0.2 g of $MgCl_2$; *p*H 7.1, with forced aeration. For glucose-limited growth, the glucose concentration was 5.5 mM and the $(NH_4)_2SO_4$ concentration was 15 mM. For nitrogen-limited growth, the glucose concentration was 28 mM and the $(NH_4)_2SO_4$ concentration was 1.5 mM. Growth was followed by measuring the turbidity of the culture at 540 nm or by estimating the number of viable cells by plate counts.

**Extraction of adenine nucleotides from E. coli.** The cells were extracted by using either cold perchloric acid or hot ethanol. The two methods gave identical results with respect to the adenine nucleotide levels obtained and were used interchangeably.

**Perchlorate treatment.** A 1-ml sample of the bacterial culture was removed through a small tube (by momentarily closing the air outlet of the culture flask) and pipetted into 0.2 ml of cold 35% $HClO_4$ within 10 sec after removal. After 15 min at 0 C, the extract was

neutralized with 0.5 ml of cold 2.6 N KOH. The denatured protein was removed by centrifugation, and the sample was assayed immediately for adenine nucleotides or stored frozen for up to 2 weeks.

**Ethanol treatment.** A 1-ml sample of the culture was rapidly injected into 1 ml of hot ethanol. After incubation in a hot-water bath (80 C) for 10 min, the sample was cooled in ice, the volume was readjusted to 2 ml with cold water, and the denatured protein was removed by centrifugation. The sample was assayed or stored frozen as above.

**Luciferase assay.** ATP was determined by means of the luciferase reaction (81) with a Luminescence Biometer (E.I. DuPont deNemours and Co). This instrument has been briefly described by Johnson et al. (44).

One vial of the crystalline luciferin-luciferase mixture supplied by DuPont was dissolved in 2.5 ml of MOPS (morpholinopropane sulfonic acid) buffer, *p*H 7.4, and 0.5 ml of glycerol. At this concentration, the enzyme mixture was completely stable for 4 days at 0 to 4 C, whereas it was rapidly inactivated in more dilute solutions.

To a reaction cuvette containing 100 μliters of the assay buffer (40 mM glycylglycine, 3 mM $MgCl_2$, *p*H 7.4) at room temperature was added 10 μliters of cold luciferin-luciferase mixture. The cuvette was placed in the Biometer, and 10 μliters of the extract was rapidly injected into the mixture. The instrument was routinely standardized against a 1 μM ATP solution. Standard ATP in a concentration range of 2 nM to 10 μM (0.02 to 100 pmoles per 10-μliter sample) gave a reproducible linear response.

The earlier part of the work was carried out with luciferin and luciferase extracted and partially purified from firefly lanterns by the method of Rasmussen and Nielsen (69) and using a Packard 2000 scintillation counter. Desiccated fireflies and lanterns were obtained from Sigma Chemical Co. and from Kurt Lehman, Cambridge, Iowa. The procedure with the commercial crystalline luciferin-luciferase and the Luminescence Biometer was about 50 times more sensitive than the earlier procedure, mainly because of a more active enzyme-substrate mixture.

**Preparing samples for the luciferase assay.** The method for luciferase assay was essentially that of Pradet (65). For ATP determinations, 200 μliters of the cell extract was added to 50 μliters of a 75 mM potassium phosphate buffer, *p*H 7.3, 15 mM in $MgCl_2$.

For ATP plus ADP determinations, 200 μliters of the cell extract was added to 50 μliters of a solution containing 75 mM potassium phosphate (*p*H 7.3), 15 mM $MgCl_2$, 0.5 mM phosphoenolpyruvate, and 20 μg of pyruvate kinase.

For total adenylate determinations, 200 μliters of the cell extract was added to 50 μliters of a solution containing 75 mM potassium phosphate (*p*H 7.3), 15 mM $MgCl_2$, 0.5 mM phosphoenolpyruvate, 20 μg of pyruvate kinase, and 25 μg of adenylate kinase.

The three mixtures were incubated at 30 C for 15 min and then held at 0 C until assayed. Adenylate kinase (Boehringer) was dialyzed against 50 mM potassium phosphate, *p*H 7.3, before use. ADP and AMP were determined by difference.

Both perchlorate and ethanol inhibit the luciferase reaction. The final concentration of perchlorate in the assay mixture was 0.26%, which caused a 25% inhibition of the reaction. This inhibition has been corrected for in the calculations.

**Determination of adenine nucleotides in the growth medium.** A sample of the growth medium was obtained by rapidly filtering an approximately 2-ml sample of the cell culture through a membrane filter (Millipore Corp.; 0.45-μm pore size, 25 mm in diameter). A 1-ml sample of the filtrate was immediately added to 0.2 ml of perchlorate, and the sample was treated as previously described. The intracellular adenine nucleotide level was determined as the difference between the adenylate level in the complete culture and the level in a sample of the medium taken simultaneously. Levels reported here are expressed as concentrations based on the culture volume. They correspond roughly to the levels (3.5 to 7 μmoles of adenine nucleotides per g of dry weight) that have been reported for *E. coli* elsewhere (34).

Glucose in the medium was determined by the glucose oxidase reaction using a Sigma no. 510 glucose kit.

Cell viability was determined by plating 1 ml of bacterial suspension ($10^7$- to $10^8$-fold diluted) on nutrient agar and counting the number of colonies formed after 24 hr of incubation at 30 C.

## RESULTS

Under all conditions investigated, the adenylate energy charge in exponentially growing cells was near 0.8. When growth stopped because of exhaustion of the carbon source in medium in which glucose was limiting, the energy charge decreased (Fig. 1). The value after cessation of growth was usually between 0.6 and 0.7, with some variation between experiments. When glu-

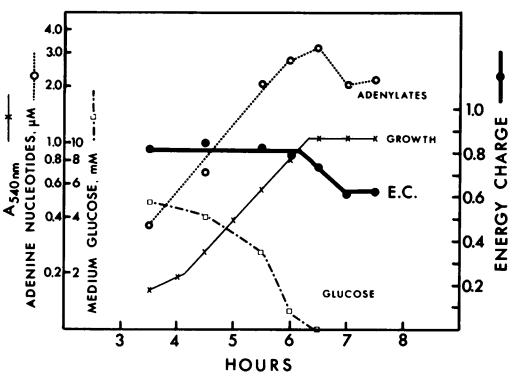


FIG. 1. *Energy charge in Escherichia coli grown on limiting glucose. Cells were grown aerobically in a 5.5 mM glucose medium, and the turbidity of the culture was followed at 540 nm (thin solid line). Zero time is time of inoculation. Upper broken line shows the total concentration of adenine nucleotides (ATP + ADP + AMP) in the culture. Heavy solid line shows the corresponding energy charge values, expressed on a linear scale as indicated on the right-hand ordinate. Lower broken line indicates concentration of glucose in the medium.*

cose was added back to the culture 45 min after cessation of growth, the value of the energy charge rose rapidly to the level characteristic of growing cultures (Fig. 2).

On cessation of growth in glucose-limited cultures, the sum of the concentrations of the metabolically active adenine nucleotides (ATP, ADP, and AMP) fell sharply. The products into which they were converted have not yet been identified. The decrease may merely reflect continued synthesis of ribonucleic acid (RNA) on a small scale for a short period. The normal pool level was restored on the addition of glucose (Fig. 2).

In cells grown in a medium in which nitrogen was limiting, there was no detectable change in the energy charge when growth stopped (Fig. 3), and the decrease in the adenylate pool was relatively small.

When aerobically growing bacteria were subjected to anaerobic conditions, there was a small but reproducible transient decrease in the energy charge accompanying a decreased growth rate (Fig. 4). There was a simultaneous slight decrease in the adenine nucleotide pool size. When aeration was resumed, the pool size rapidly rose to the original aerobic level, and the energy charge was unaffected.

The results of a complementary experiment are shown in Fig. 5. Anaerobically growing cells were aerated for 50 min, after which anaerobic conditions were restored. The energy charge values showed no change during these transi-

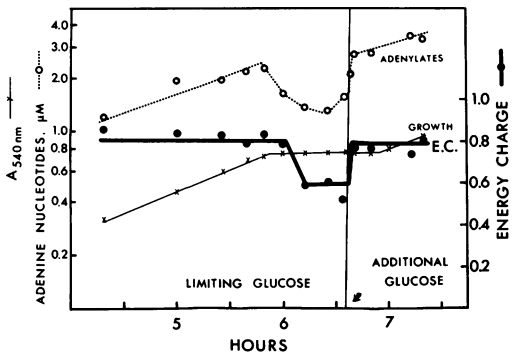


FIG. 2. *Energy charge in Escherichia coli grown on limiting glucose followed by addition of more glucose. Cells were grown aerobically on a 5.5 mM glucose medium as described in the legend to Fig. 1. At 50 min after cessation of growth, glucose was added to restore a glucose concentration of 5.5 mM. Broken line shows total concentration of adenine nucleotides (ATP + ADP + AMP) in the culture; heavy solid line shows corresponding energy charge values.*

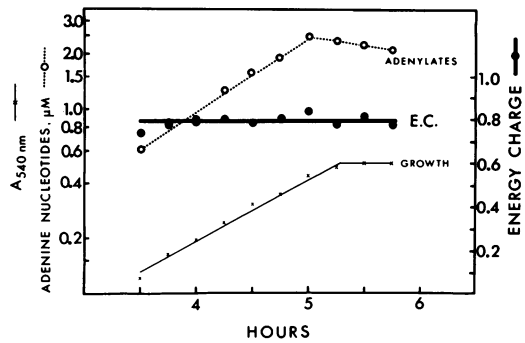


FIG. 3. *Energy charge in Escherichia coli grown on limiting nitrogen. Cells were grown aerobically in medium containing 28 mM glucose and 1.5 mM $(NH_4)_2SO_4$. Turbidity measured at 540 nm is shown by the thin solid line, concentration of total adenine nucleotides (ATP + ADP + AMP) by the broken line, and corresponding energy charge values by the heavy solid line.*

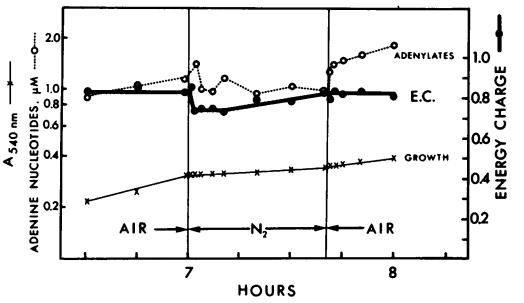


FIG. 4. *Energy charge in aerobically growing Escherichia coli; effect of temporary anaerobiosis. Cells were grown aerobically on excess glucose and ammonium sulfate. The culture was made anaerobic for 40 min during early exponential phase, after which aeration was resumed. Thin solid line shows turbidity of the culture measured at 540 nm. Total concentration of adenine nucleotides (ATP + ADP + AMP) is shown by the broken line. Heavy solid line gives corresponding energy charge values.*

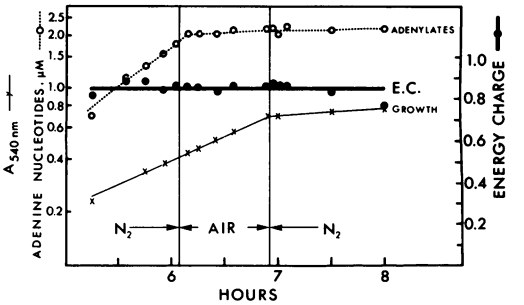


FIG. 5. *Energy charge in anaerobically growing Escherichia coli; effect of aeration. Cells were grown anaerobically on excess glucose and ammonium sulfate. During early exponential phase, the culture was aerated for 50 min, after which anaerobic conditions were restored. Turbidity of the culture as measured at 540 nm is shown by the thin solid line. Heavy line shows corresponding energy charge values expressed on a linear scale as indicated on the right-hand ordinate.*

tions, and the adenine nucleotide pool size was affected only slightly.

*E. coli* cells remain viable for at least 60 to 80 hr in a glucose-mineral medium that has been adequately buffered so that the *p*H of the medium remains close to neutrality. When energy charge determinations were made in stationary-phase cells, it was discovered that a relatively large amount of adenine nucleotide was secreted into the medium, mainly as AMP. This is in agreement with previous reports of release of nucleotides into the medium by stationary-phase bacteria, presumably as a consequence of RNA degradation (18). It was further discovered that, during the latter part of the stationary phase, the medium contained enzyme activities that could rapidly destroy the adenine nucleotides present in the medium. For instance, almost 50% of the AMP in filtered medium from stationary-phase cultures disappears within 15 min. It is therefore essential to filter rapidly and inactivate extracellular enzymes with perchloric acid promptly in order to obtain accurate values for nucleotides in the medium, which are needed for calculation of intracellular levels. Marked variations were observed between cultures in the amount of AMP in the medium. It is not clear whether these differences result from differential rates of excretion of AMP or of enzymes that remove AMP. Despite those differences, the pattern of change with time in intracellular energy charge was basically similar in all cultures observed.

When an *E. coli* culture enters stationary phase, the apparent energy charge of the total culture falls quite rapidly (Fig. 6), whereas the intracellular energy charge decreases gradually to near 0.5 and then falls relatively steeply. The number of viable cells does not decrease during the first phase of gradual decline in energy charge, but cell death coincides with the final steep fall in charge.

When a 20-hr culture was filtered and the cells were used to inoculate a volume of fresh medium 50 times that of the original culture, the energy charge rose during the first 15 to 20 min to about 0.8 before resumption of growth (Fig. 7). Inoculation was usually carried out by transfer from an unfiltered 20-hr culture to 100 times the volume of the inoculum. In such cases, the apparent energy charge of the culture was usually around 0.4 or 0.5 during the first hour or two because of extracellular adenine nucleotides (mainly AMP) carried over with the stationary-phase inoculum. The corrected intracellular energy charge values obtained in such experiments agree with the results presented in Fig. 7.

Cole and co-workers (29) reported that the ATP pool in *E. coli* decreases by about 50%

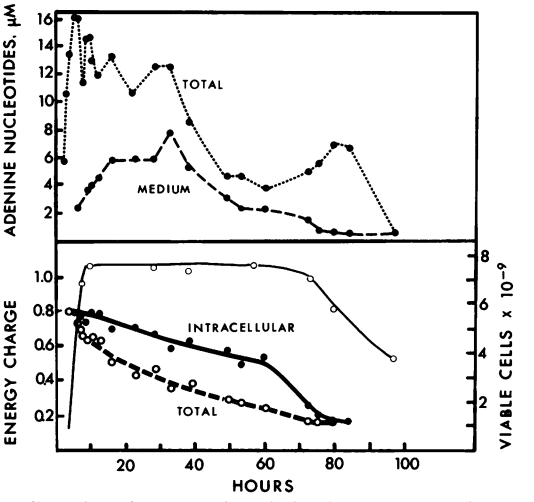


FIG. 6. *Adenine nucleotide levels and energy charge in Escherichia coli during stationary phase. Cells were grown aerobically in medium containing excess (28 mM) glucose. The phosphate buffer concentration was 0.22 M, twice that specified in Materials and Methods. The pH of the culture remained above 6.7 throughout the period represented. Upper curves show total concentrations of adenine nucleotides (ATP, ADP, and AMP) in the total culture and in medium from which cells had been removed rapidly by filtration. Thin solid line indicates number of viable cells as estimated by plate count. Heavy solid line shows energy charge of the cells, calculated by correcting for nucleotides excreted into the medium. Heavy broken line shows apparent energy charge values of the total culture (cells plus medium).*

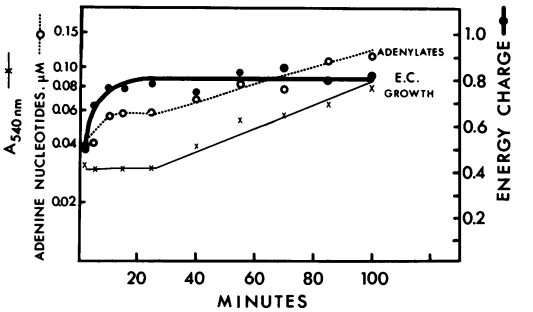


FIG. 7. *Energy charge in Escherichia coli during initial lag phase and early exponential phase. A 2-ml sample of a 20-hr culture (energy charge value was 0.70) was filtered, and the filter bearing the cells was added to 100 ml of fresh medium containing excess glucose. Turbidity was measured at 540 nm. Total concentration of adenine nucleotides (ATP, ADP, and AMP) is given by the broken line and corresponding energy charge values by the heavy solid line.*

when the cells are centrifuged. We have found that there is a time-dependent variation in the energy charge values and the adenylate pool after resuspension of cells after centrifugation or

membrane filtration. Exponentially growing aerobic cultures of *E. coli* (energy charge values about 0.8) were filtered and resuspended in the same volume of fresh medium in the absence and presence of glucose. During the course of filtration (10 to 30 min), the energy charge dropped to about 0.5, and the adenine nucleotide pool was reduced by 50%. When the cells were resuspended in glucose-free medium, the energy charge value rose rapidly to about 0.8 before again decreasing to about 0.6 (Fig. 8). Presumably this transient rise corresponds to the exhaustion of a small endogenous energy supply. The behavior of these cultures is followed for a longer period in Fig. 9. On this scale, the initial fluctuations observed in Fig. 8 have been omitted. In the culture lacking glucose, a charge value of about 0.6 was retained for as long as the cells remained fully viable (Fig. 9). Cell death was accompanied by a rapid decrease in the energy charge.

The adenine nucleotide pool size likewise undergoes a rapid increase during the first 2 or 3 min after resuspension in a glucose-free medium. This is followed by an equally rapid decrease, until a new steady state is reached. The large

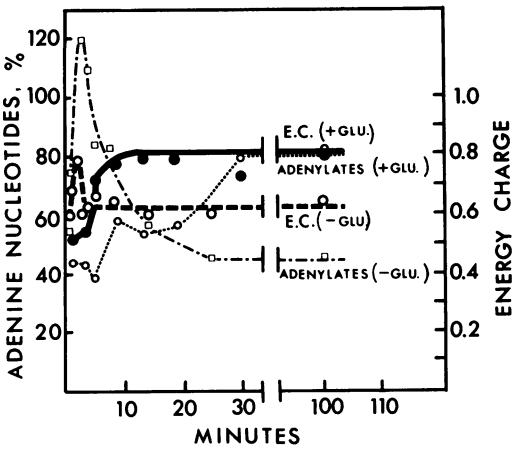


FIG. 8. *Energy charge in filtered Escherichia coli cells after resuspension in fresh medium in the absence and presence of glucose and nitrogen. Samples (150 ml) of aerobically growing E. coli in mid-exponential phase ($A_{540\ nm}$ = 0.700, energy charge = 0.81) were filtered, and the filters were resuspended in 150 ml of fresh medium lacking either glucose or nitrogen. Heavy broken line shows energy charge in the culture after resuspension of the cells in a glucose-free medium. Heavy solid line shows energy charge of the cells after resuspension in a 28 mM glucose medium lacking nitrogen. Broken lines show total concentrations of adenine nucleotides (ATP, ADP, and AMP) in the two suspensions. Concentrations are expressed as percentages of the level found in the original growing culture before filtration.*

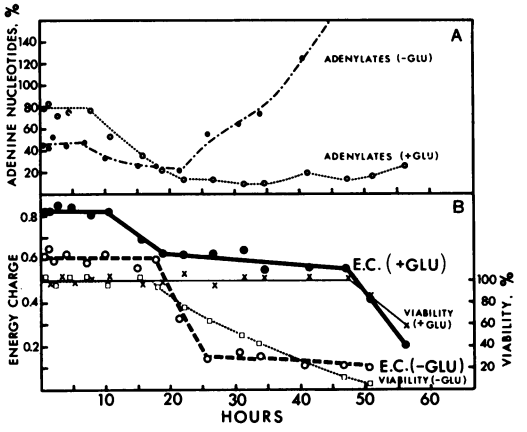


FIG. 9. *Energy charge and viability of filtered Escherichia coli cells after resuspension in fresh medium in the absence and presence of glucose. This figure presents results, obtained at later times, from the experiment of Fig. 8. Experimental data obtained during the first 30 min (presented in Fig. 8) have been omitted here for simplicity. (A) Total concentrations of adenine nucleotides (ATP, ADP, and AMP) in the two cultures expressed as percentages of the level in the original culture. Only insignificant amounts of adenine nucleotides were observed in the medium in this experiment. (B) Thin solid line shows viability of the cells resuspended in medium containing 28 mM glucose and no nitrogen, expressed as percentage of the number of viable cells present in the original culture. Thin broken line shows viability of the cells resuspended in a glucose-free medium. Heavy broken and heavy solid lines show the energy charge values of the cells resuspended in the absence and presence of glucose, respectively.*

increase in pool size observed when cell death begins may be caused by degradation of RNA.

The initial increase in energy charge is always slower when the cells are resuspended in a glucose-containing medium than when the medium contains no energy source (Fig. 8). This delay may result from the uptake of an amount of glucose large enough that the hexokinase reaction puts a heavy drain on the ATP supply. However, in the presence of glucose, the energy charge value of the cells remains near 0.8 for an extended period (about 12 hr in Fig. 9), presumably until the glucose supply is exhausted, after which it slowly decreases to about 0.55 (Fig. 9). Again, the final sharp drop in energy charge coincides with the onset of extensive cell death.

The initial sharp fluctuation in adenylate pool size observed when filtered cells are resuspended in glucose-free medium does not occur when glucose is present in the medium. The pool size gradually increases during the first 30 min to a level that is maintained for several hours. This is followed by a gradual decrease in the pool size until only about 10% of the original adenine nu-

cleotides remain. The energy charge remains above 0.55 during this period.

## DISCUSSION

**Relation of adenylate energy charge to growth.** The results reported here suggest that, during normal growth of *E. coli*, the adenylate energy charge value is strongly poised at a value of about 0.8. An equivalent statement is that growth appears to be possible only when the energy charge is at 0.8 or above. Maintenance of viability, but not growth, seems possible at charge values between about 0.8 and 0.5. When removed from starving cultures with energy charge values in this range, all cells were found to be capable of forming colonies when plated on complete medium (Fig. 6 and 9). Energy charge values below 0.5 are apparently incompatible with maintenance of the minimal level of homeostasis required for viability. After declining to this value, the charge falls more steeply, and concurrently the number of cells capable of recovery when transferred to complete medium decreases rapidly.

Loss of viability at charge values below about 0.5 is not surprising. Most of the energy charge response curves obtained for regulatory enzymes in vitro (for review, see reference 6) are steep in the region above about 0.75 but become nearly horizontal at charge values below 0.6 or 0.5. In some cases, the curves even have the "wrong" (antistabilizing) slope at low values of charge. For example, the activity of phosphofructokinase appropriately decreases sharply with increase in energy charge at high charge values (77), but, because ATP is a reactant, the reaction velocity must also decrease to zero at zero energy charge. Since metabolic regulation is necessary to life, it thus seems inevitable that a decrease in energy charge below about 0.5 must lead to a lethal disintegration of the cellular economy. Probably such low values of charge will be found to be compatible with life only in dormant structures such as spores or seeds, where enzymes have been rendered inactive by dehydration or otherwise.

At present, our determinations of energy charge have an uncertainty of about 0.06. Thus we are able to observe only relatively large changes caused by such drastic metabolic events as exhaustion of carbon source or depletion of endogenous reserves during stationary phase. The range of the energy charge values in growing *E. coli* cells is probably too narrow to permit detection, by the assay used here, of the variations that might be expected (for example, possible variation of charge during the cell cycle in synchronized cultures, or variation associated with growth rate, as in a chemostat). Even the transition from aerobic to anaerobic conditions appears to cause a temporary decrease of only about 0.1 in energy charge, but this change is associated with complete cessation of growth.

Studies by Somlo on mutant $op_1$ of bakers' yeast (*Saccharomyces cerevisiae*) supply a striking illustration of the importance of energy charge to growth (78). This mutant resembles the well-known "petite" yeast mutants in its ability to grow fermentatively with glucose as substrate and its inability to use a respiratory substrate such as ethanol for growth. Unlike the petite strains, however, mutant $op_1$ possesses an apparently normal complement of cytochromes, oxidizes ethanol, and even carries out electron transport phosphorylation of ADP at the expense of ethanol oxidation. The intracellular level of ATP in the mutant when oxidizing ethanol is similar to that of the wild-type parent under the same conditions. Yet the mutant not only fails to grow with ethanol as carbon and energy source but is totally unable to synthetize proteins, as indicated by lack of incorporation of labeled valine into proteins. The apparent explanation of this inability to grow or to synthesize macromolecules was supplied by the observation that the level of AMP is several times higher in the mutant than in the wild-type strain. Thus the energy charge in cells oxidizing ethanol was 0.86 in the wild-type strain but only 0.67 in the mutant. The mutant, although capable of maintaining a normal level of ATP by electron transport phosphorylation, is not able to maintain a normal value of the adenylate energy charge. The immediate effect of the mutation has not yet been identified, and the mutant differs from the wild-type strain in various other characteristics and metabolic responses as well as in the value of its adenylate energy charge. Nevertheless, it seems very likely that inability of the mutant to grow and to synthesize macromolecules results from the low value of its intracellular energy charge.

It is of interest that the value of the energy charge maintained by the yeast mutant when oxidizing ethanol is well within the range in which we observed *E. coli* cells to survive but to be incapable of growth. It should also be noted that several enzymes that have been shown to respond in vitro to the value of the energy charge are insensitive, over a considerable range, to the absolute level of ATP or other nucleotides (5, 9, 10). The behavior of yeast mutant $op_1$ strongly suggests a similar response in intact living cells; that is, it is not the concentration of ATP but the ratio of this concentration to those of ADP and AMP that determines the rates of ATP-requiring reactions.

**Intracellular energy charge values.** The results presented in this paper and those reported by Somlo (78) confirm, for a bacterial species and a unicellular eukaryote, the prediction, based on the response curves in vitro of enzymes from ATP-regenerating and ATP-utilizing sequences (5–7, 10), that the energy charge of intact cells during active metabolism should be stabilized in the vicinity of 0.8 to 0.85. A further test of this prediction is available in the literature, since an energy charge value may be calculated from any determination of the relative concentrations of ATP, ADP, and AMP in vivo. A number of such analyses reported before 1957 are tabulated in the *Biochemists' Handbook* (51). No significant pattern of differences between organisms or tissues is apparent; variations in the value of energy charge calculated from the tabulated values seem to arise mainly from differences in the analytical methods employed. Energy charge values based on 24 studies using chemical methods range from 0.21 to 0.94, with 19 below 0.7 and only 5 above. In contrast, 11 determinations using enzymatic methods lead to energy charge values ranging from 0.37 to 1.0, with 3 below 0.7 and 8 above.

Bomsel and Pradet (17) tabulated more recent estimates of adenine nucleotide levels in mammalian tissues. Of these, six correspond to energy charge values below 0.75 and 14 above. (The ratio $\epsilon/\Sigma$ tabulated by Bomsel and Pradet is twice the adenylate energy charge defined here.)

Energy charge values based on all adenine nucleotide determinations that we have encountered in the literature, other than those tabulated in the *Biochemists' Handbook* or by Bomsel and Pradet (17), are listed in Table 1. Some of these determinations were made with great care, and others were carried out rather casually, as adjuncts to other studies. Nevertheless, the preponderance of entries between 0.75 and 0.9 is striking, and it seems valid to conclude that values are converging in this range as analytical methods improve.

Mammalian tissues are usually protected against extreme changes in external conditions, and they show uniformly high energy charge values, in the range between 0.75 and 0.9. The amount of variation observed as a result of starvation, muscle contraction, or similar stresses usually does not exceed 0.1 energy charge unit. Large variations are observed only under nonphysiological conditions such as exposure of the whole animal to anaerobic conditions (12), inhibition of oxidative phosphorylation (27, 56, 86), or perfusion of liver with fructose (95).

Most of the energy charge values reported for green plants are lower than those for other metabolically active tissues. The physiological significance of this difference is not clear. Perhaps the extensive vacuolization of leaf cells is relevant; only a relatively low concentration of AMP in the vacuolar sap would be required to reduce the apparent energy charge to the observed values, even if the true cytoplasmic values were similar to those observed for other organisms. The energy charge in leaves decreases rapidly by approximately 0.2 units after a change from light to dark. When light is restored, the energy charge value quickly readjusts to the original level (74).

Bacterial spores have extremely low energy charge values (76), whereas the values observed in vegetative bacterial cells usually agree satisfactorily with our results for growing *E. coli* cells. Similarly, dry, mature seeds have low charge values but, when the tissue becomes metabolically active, the energy charge is found to be in the normal range for green plants (1, 19, 20, 66). Indeed, germinating seeds may have exceptionally high charge values. Pradet (66) showed that the energy charge in germinating lettuce seeds reached a value of about 0.97 under air but decreased as the level of $O_2$ was decreased, reaching a value as low as 0.1 in the absence of $O_2$. This interesting result seems readily rationalized. Charge values determined on whole seeds must reflect mainly the situation in the storage tissue. Since this tissue has no biological future, being specialized for delivery of nutrients to the developing embryo, there is no apparent reason for its energy charge to be stabilized. Both the extremely high values obtained in the presence of $O_2$ and the very low levels under anaerobic conditions are consistent with lack of energy charge control in the endosperm.

Only a few of the energy charge values calculated for microorganisms are sufficiently unrepresentative to require comment. We can suggest no explanation for the very low values reported for yeast by Polakis and Bartley (64) or for the report from the same laboratory that the energy charge of an anaerobic culture decreased on aeration (25). These results are incompatible with those of Somlo (78), Kopperschläger et al. (46), Barwell and Brunt (13), and with our own unpublished observation of an energy charge of about 0.80 in growing yeast.

The value of 0.36 reported for *Bacillus licheniformis* is based on analyses performed on cells after centrifuging and washing (49). The levels of metabolites may have changed considerably during this treatment, as is suggested by the report of Cole et al. (29) and by our determinations of adenine nucleotide levels in *E. coli* after membrane filtration. The values of 0.57 and 0.35

TABLE 1. *Adenylate energy charge values of various organisms and tissues*

| Organism or tissue | Comments | Energy charge | Reference |
|---|---|---|---|
| *Escherichia coli* | Aerobic, exponential growth, various media | 0.90–0.94 | 34 |
| *Aerobacter aerogenes* | Starved stationary phase | | |
| | Aerobic | 0.57 | 80 |
| | Anaeobic | 0.35 | |
| *Klebsiella aerogenes* | $O_2$ pressure | | |
| | 2–5 mm Hg | 0.81–0.84 | 40 |
| | 0 | 0.65 | |
| *Azotobacter vinelandii* | Exponential growth | 0.83 | 50 |
| *Methanobacterium* | Maximal $CH_4$ production | 0.67[a] | 71 |
| *Bacillus megaterium* | Vegetative cells | >0.79 | 76 |
| | Spores | 0.09 | |
| *B. cereus* | Spores | 0.08 | |
| *B. subtilis* | Spores | 0.08 | |
| *B. licheniformis* | Stationary, presporulation; centrifuged and washed | 0.36 | 49 |
| *Chromatium D* | Thiosulfate-grown | | |
| | Dark, anaerobic | 0.78 | 38 |
| | Dark, aerobic | 0.74 | |
| | Light, anaerobic | 0.91 | |
| | Light, aerobic | 0.91 | |
| *Rhodospirillum rubrum* | Various media, etc. | | |
| | Dark, anaerobic | 0.32–0.59 | 75 |
| | Dark, aerobic | 0.65–0.80 | |
| | Light, anaerobic | 0.56–0.80 | |
| | Light, aerobic | 0.77–0.82 | |
| *Rhodopseudomonas spheroides* | Light, anaerobic[b] | 0.35–0.80 | 33 |
| *Tritricomonas foetus* | Anaerobic | 0.90 | 24 |
| | Aerated 15 min | 0.83 | |
| *Saccharomyces cerevisiae* | | 0.84–0.93 | 16 |
| *S. cerevisiae* | | 0.86[c] | 78 |
| *S. cerevisiae* | Aerobic, galactose | 0.26–0.44 | 64 |
| | Aerobic, glucose | 0.20–0.40 | |
| *S. cerevisiae* | Aerobic | 0.26 | 25 |
| | Anaerobic | 0.65–0.70 | |
| *S. cerevisiae* | Resting cells | | |
| | External *p*H 2.0 | 0.82 | 13 |
| | External *p*H 4.5–8.5 | 0.91 | |
| *S. cerevisiae* | Starved | | |
| | Anaerobic | 0.47 | 46 |
| | Aerobic | 0.64 | |
| | Starved, glucose added | | |
| | Anaerobic[d] | 0.78–0.82[a] | |
| | Aerobic[d] | 0.86–0.89[a] | |
| *Dictyostelium discoideum* | 20 C light | 0.53 | 45 |
| | 20 C dark | 0.72 | |
| | 4 C light | 0.80 | |
| | 4 C dark | 0.83 | |

TABLE 1—*Continued*

| Organism or tissue | Comments | Energy charge | Reference |
|---|---|---|---|
| Barley leaves | | 0.60 | 36 |
| Pea leaves | | 0.64 | |
| Bean leaves (excised) | Petioles | 0.87–0.95 | 22 |
| | Leaf laminae | 0.80 | |
| Wheat leaves | Dark, $N_2$ | 0.21 | 17 |
| | Dark, air | 0.61 | |
| | Light, $N_2$ | 0.48 | |
| | Light, air | 0.60 | |
| Bean leaves | | 0.44 | 3 |
| Pinto bean leaves | Young | 0.73 | 88 |
| | Mature | 0.69 | |
| | Senescent | 0.76 | |
| Beet leaves | Light[e] | 0.57[a] | 74 |
| | Dark[e] | 0.37[a] | |
| *Coleus shirensis* leaves | Light[e] | 0.40–0.53[a] | 74 |
| | Dark[e] | 0.19[a] | |
| Spinach leaves | Light[e] | 0.56–0.69[a] | 74 |
| | Dark[e] | 0.43–0.51[a] | |
| Tomato leaves | | 0.63 | 72 |
| Sugar beet leaves | Whole leaf | 0.68 | 61 |
| | Petioles | 0.43 | |
| | Vascular bundles | 0.39 | |
| Wheat | Whole plant | 0.62 | 14 |
| Barley | Aerial portion | 0.54 | 15 |
| Oat | Aerial portion | 0.76 | 15 |
| Pea roots | Control | 0.83 | 41 |
| | High salt medium | 0.73 | |
| Jerusalem artichoke tubers | | 0.61 | 84 |
| Lemon fruits | Various fruit sizes | 0.57–0.73 | 2 |
| Lettuce seeds | Germinating[c] | | |
| | 10–20% $O_2$ | 0.97 | 66 |
| | 0–1% $O_2$ | 0.11–0.35 | |
| Pea seeds | Mature, dry | 0.28 | 19 |
| Pea seeds | Mature, dry | 0.24 | 20 |
| | End of imbibition phase (16 hr) | 0.49 | |
| | Protrusion of radicals (40 hr) | 0.58 | |
| *Vicia faba* | Seeds | 0.47 | 1 |
| | 1–3 weeks | 0.58 | |
| | 4 weeks | 0.37 | |

TABLE 1—*Continued*

| Organism or tissue | Comments | Energy charge | Reference |
|---|---|---|---|
| Wheat grains | 12 days after anthesis | 0.87 | 43 |
| | 32 days after anthesis | 0.71 | |
| Sycamore cells | Cell-culture | | |
| | Lag phase | 0.73 | 21 |
| | Exponentially growing | 0.66 | |
| | Stationary phase | 0.81 | |
| Sea urchin eggs | 2–3 Min after fertilization | 0.86[a] | 32 |
| Earthworm muscle | Resting | 0.87 | 70 |
| | 20-Min electrical stimulation | 0.65 | |
| Squid muscle | | 0.88 | 73 |
| Electric eel | Electric organ | | |
| | Beginning of discharge | 0.94[a] | 92 |
| | End of 64-sec discharge | 0.91[a] | |
| | 1 Min after discharge | 0.79[a] | |
| | 5 Min after discharge | 0.94[a] | |
| Chick fibroblasts | Various conditions in cell culture | 0.87–0.95 | 28 |
| Chick brain | Control diet | 0.85 | 47 |
| | Galactose diet | 0.76 | |
| Rat brain | | 0.93 | 59 |
| Mouse brain | Adult | 0.81 | 52 |
| | 10-Day-old | 0.87 | |
| Rat embryo | 14–15 Day | 0.85 | 26 |
| Rat muscle | Killing method | | |
| | Pentobarbital | 0.96 | 54 |
| | Ether | 0.89 | |
| | Decapitation | 0.88 | |
| Rat muscle | Resting | 0.93[a] | 63 |
| | 5–20 Sec electrical stimulation | 0.93[a] | |
| Rat platelets | | 0.88 | 30 |
| Human erythrocytes | | 0.91 | 55 |
| Human erythrocytes | | 0.93 | 82 |
| Rabbit reticulocytes | Air, + glucose | 0.93[a] | 35 |
| | $N_2$, first 20 min | 0.94[a] | |
| | $N_2$, after 80 min | 0.35[a] | |
| Frog heart | | 0.82 | 4 |
| Rat heart | Control | 0.75 | 68 |
| | After antivitamin K injection | 0.71 | |
| Rat heart | Control | 0.90 | 58 |
| | Glucagon perfused | 0.90 | |
| | Anaerobic | 0.78 | |

TABLE 1—*Continued*

| Organism or tissue | Comments | Energy charge | Reference |
|---|---|---|---|
| Rat heart | Control | 0.90 | 89 |
| | Acetate perfused | 0.93 | |
| | Pyruvate perfused | 0.94 | |
| Rat kidney slices | | 0.74 | 60 |
| Rat kidney cortex slices | Control | 0.80 | 86 |
| | Starved 48 hr | 0.84 | |
| | Cyanide poisoned | 0.41–0.46 | |
| Rat kidney slices | | 0.59 | 31 |
| Hamster brown adipose tissue | Fed | 0.71–0.80 | 90 |
| | Fasted | 0.74–0.81 | |
| Rat liver | Fetus | 0.71 | 11 |
| | Newborn | 0.86 | |
| Rat liver | | 0.87 | 67 |
| Rat liver | Control | 0.69 | 87 |
| | Starved 48 hr | 0.62 | |
| | High glucose diet | 0.76 | |
| Rat liver | Regenerating | | |
| | 0 Hr after partial hepatectomy | 0.73 | 57 |
| | 6–24 Hr | 0.60 | |
| Rat liver | | 0.56 | 31 |
| Rat liver | Control | 0.80 | 95 |
| | Fructose perfused 10 min | 0.61 | |
| | Fructose perfused 40 min | 0.76 | |
| Rat liver | Adult | 0.83 | 62 |
| | Newborn | 0.86 | |
| | Fetus | 0.78 | |
| Rat liver | Control | 0.85 | 23 |
| | Glycerol injection | 0.75 | |
| | Riboflavin-deficient | 0.76 | |
| Rat liver | Control | 0.52 | 83 |
| | Thyroidectomized | 0.64 | |
| | Thyroidectomized, + thyroxin | 0.55 | |
| Rat liver | Control | 0.85 | 79 |
| | Starved 48 hr | 0.79 | |
| | Alloxan diabetes | 0.80 | |
| Rat liver | | 0.80 | 48 |
| Rat liver[f] | Control | 0.88[f] | 91, 93, 94 |
| Rat liver | Young animals exposed to | | |
| | 100-12% air | 0.82 | 12 |
| | $N_2$ | 0.59 | |
| Guinea pig liver | Control | 0.85 | 37 |
| | Starved 96 hr | 0.87 | |
| Rat liver mitochondria | Control | 0.60–0.62[a] | 27 |
| | Valinomycin | 0.68–0.72[a] | |
| Novikoff ascites tumor | Control | 0.81 | 56 |
| | Dinitrophenol | 0 | |
| | 15 Min after dinitrophenol | 0.74 | |
| Ascites tumor | | 0.92 | 54 |
| Ascites tumor | Control | 0.90 | 96 |
| | Glucose | 0.93 | |
| | Anaerobic, – glucose | 0.12 | |
| | Anaerobic, + glucose | 0.93 | |

TABLE 1—*Continued*

| Organism or tissue | Comments | Energy charge | Reference |
|---|---|---|---|
| Ascites tumor | Control | 0.89[a] | 42 |
| | 0–3 Min after glucose addition[g] | 0.57–0.86[a] | |
| Krebs ascites tumor | Glucose | 0.79 | 39 |
| Ehrlich ascites tumor | | 0.84 | 85 |
| Zazhdal ascites tumor | Control | 0.79 | 31 |
| | Glucose | 0.83 | |
| Zazhdal hepatoma | Control | 0.70 | 31 |
| | Glucose | 0.68 | |
| Sarcoma 37 | Control | 0.73 | 31 |
| | Glucose | 0.74 | |
| Ovarian cancer cells | Control | 0.64 | 31 |
| | Glucose | 0.64 | |

[a] Adenine nucleotide concentrations were estimated from graphs for the purpose of energy charge calculations.
[b] Large oscillations in energy charge during exponential growth.
[c] See discussion in text.
[d] Transient drop in energy charge on addition of glucose, probably because of hexokinase activity.
[e] Nucleotides measured in isolated chloroplasts from leaves exposed to light or dark conditions. Energy charge dropped sharply within 20 sec when light was turned off.
[f] In the three papers cited, four control values (0.88, 0.88, 0.84, 0.90) averaged 0.88; 14 values were given for liver perfused with various substrates. Of these, 13 were between 0.8 and 0.9; the other was 0.72.
[g] Large oscillations in energy charge following glucose addition; however, ATP was measured in samples different from those used for AMP and ADP measurements.

calculated for *Aerobacter aerogenes* (80) were obtained on starved cells. Our results show that starved *E. coli* cells sometimes excrete considerable amounts of AMP into the medium. If *A. aerogenes* behaves similarly, the actual intracellular charge values may have been considerably higher than those calculated for the total culture.

We have pointed out elsewhere (8) that the role of the adenine nucleotides in stoichiometrically coupling all metabolic sequences requires that the ratio of their concentrations (which may be specified by the energy charge) be maintained at a relatively constant value if metabolic homeostasis is to be maintained. The responses of regulatory enzymes to experimental variations in energy charge in vitro led to the prediction (5–7, 10) that this stabilized value of the energy charge should be near 0.85. The observations on *E. coli* during exponential growth and under nutritional stress reported in this paper, together with the energy charge values for a variety of organisms and tissues calculated from published analytical values, confirm these predictions and supply the final evidence needed to establish the central importance of the adenylate energy charge in correlating metabolic sequences and maintaining the metabolic stability necessary for life.

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